Goldschmidt 2026 Abstract
https://doi.org/10.46427/gold2026.34485

Data-driven identification of controls on microbially induced and abiotic carbonate mineralization.

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Abstract

Microbial biomineralization at mineral–organic–microbe interfaces regulate carbonate formation and preservation, influencing carbon turnover and mineral stabilization. Microbially induced carbonate precipitation (MICP) by *Sporosarcina pasteurii* has attracted considerable interest for applications in soil stabilization, metal sequestration, and sustainable construction. However, linking microbial processes to mineral products remains challenging because crystallization responds nonlinearly to solution chemistry, temperature, and biological mediation. A key limitation in the field is the inability to control carbonate polymorphism, size, and crystal habit parameters that influences mineral performance in different applications and are currently tuned empirically. Here, we develop and validate a high-throughput screening platform integrating microscopy-based image analysis, machine learning (ML), and response surface methodology (RSM) to identify abiotic and genetic controls on carbonate mineral formation.

Carbonate precipitation was investigated in 96-well plates under abiotic and biotic conditions. RSM identified main effects and interactions, with total particle area selected as the primary response. In abiotic systems, particle area showed a non-linear dependence on calcium concentration, increasing to an optimum before declining. In contrast, under biotic conditions, increasing calcium concentration strongly increased total particle area, highlighting Ca^{2+} availability as a dominant control on biomineralization. Calcium source and temperature had no significant effects, and tested variables acted independently. Morphological classification was performed using a random forest ML model trained on seven particle types identified by microscopy images. In abiotic systems, at 25-50 mM $CaCl_2$ concentrations new particle types were observed whereas at lower (5-10 mM) and higher (500 mM) concentrations single type was dominant. In biotic systems diverse particle morphologies were observed across different concentrations. Evenness analysis further showed that biological mediation stabilizes multiple crystal habits rather than allowing dominance by a single morphology.

Building on this framework, we initiated screening of *S. pasteurii* mutant libraries generated via chemical mutagenesis using EMS (ethyl methanesulfonate at 1 mg L^{-1}). Seventy-one mutant colonies are currently being phenotyped using the same pipeline to identify strains with altered mineralization behaviour. Overall, this work delivers both a validated analytical framework and foundational insight into controlling biologically driven carbonate mineral formation relevant to biomineralization and biogeochemical cycling.